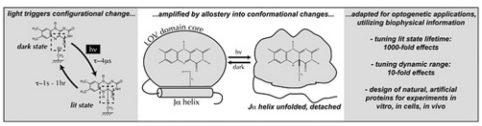
Many biological processes are regulated by environmental control of protein conformation. This is commonly achieved by sensory protein domains that bind both protein targets and environmentally-sensitive cofactors, using these compounds to trigger allosteric changes in the sensory domain. These general principles are exemplified by photosensory proteins, several of which have been amenable to biophysical study and subsequent use in applied settings.
Here I will present our work in this area, where we have used solution NMR spectroscopy and other biophysical approaches to study the signaling mechanisms of flavin-based photoreceptors that sense blue light. We have principally focused on Light-Oxygen-Voltage domains, a group of small (~120 aa) modules from plant and bacterial photosensors. These domains control intra- and intermolecular protein/protein interactions in response to photochemically-driven bond formation between protein and flavin groups. This event generates conformational changes that disrupt inhibitory protein/protein interactions, activating downstream effectors.
Combining experimental and computational approaches, we have quantitatively described how alterations in protein/cofactor interactions perturb the structural and functional activation of these proteins. In turn, this has laid the foundation for us to manipulate various photochemical properties of these sensors, improving their utility in optogenetic applications.



204-Wkshp
Channelrhodopsin: Ideas about Gating and Ion Transport
Peter Hegemann[1], Dietrich Gradmann[2], Franziska Schneider[1].
[1]Humboldt University, Berlin, Germany, [2]Universität Göttingen, Göttingen, Germany.
Channelrhodopsins (ChRs) are directly light-gated ion channels in halo- and glycophytic Chlorophyceae where they serve as sensory photoreceptors for behavioral responses (phototaxis). ChRs are used for the depolarization of selected cells in neural networks of tissues and living animals to gain information about the brain function. The operation of ChRs is determined by two fundamental functions: gating and transport. Within the voltage- pH-, time- and light-sensitive gating function (photocycle), an early and a late conductance with different transport properties were identified. The voltage- and substrate-sensitive transport function of the early conductance can quantitatively be described by enzyme kinetics, where $H+ > Na+ > K+ > Ca2+ > Mg2+$ are translocated competitively. Experiments with specific mutants, and 3D models based on structures of other microbial proteins, allow preliminary assignments of specific ChR functions to structural details.

205-Wkshp
A Light-Based Feedback Control System for Generating User-Defined Intracelullar Signaling Dynamics
Jared Toettcher, Delquin Gong, Wendell A. Lim, **Orion Weiner**.
University of California, San Francisco, San Francisco, CA, USA.
As we enter the era of systems biology, in which we are trying to unravel how complex biological systems function, we are in dire need of methods to apply quantitative inputs at specific signaling nodes. We recently developed an approach to spatially and temporally control signaling in mammalian cells with light. Here, we report a method for applying custom signaling inputs using feedback control of this optogenetic protein-protein interaction (PhyB and PIF). With our approach, time-varying inputs, which have been applied extracellularly to dissect sensory signaling cascades, can now be applied to intracellular signals. Titratable control at multiple signaling nodes can be used to ‘‘walk down’’ a pathway to identify sources of ultrasensitivity or points of feedback connection. Finally, our technique can be used to disconnect feedback circuits without changing the architecture of signaling networks by clamping light-gated inputs against cellular changes in activity. Our approach will enable a new generation of precision perturbative experiments that could revolutionize our understanding of biological systems.

206-Wkshp
Conditional Control of Protein Activity using Light-Induced Dimerizers
Chandra Tucker.
Department of Pharmacology, University of Colorado Denver, Aurora, CO, USA.
While chemical tools that allow control of protein activities and interactions have been in use for a number of decades, a recently emerging field centers on the use of light for protein control, allowing spatial, temporal, and reversible control of protein activity. Light-responsive protein modules that mediate protein dimerization in response to light can be linked to target proteins, such that activity of target proteins can be conditionally modulated by light. One such light-mediated dimerization module is based on a blue-light dependent interaction of Arabidopsis cryptochrome (CRY2) and CIB1 (Kennedy et al., 2010). Here, I will discuss the basic properties and uses of the CRY2-CIB1 dimerizer modules, providing examples of use controlling protein localization and protein activity with light. I will also discuss the use of pulsed LED light systems, that help prevent light-dependent toxicity that can result from extended light exposure.

Workshop: Biofuels

207-Wkshp
To Be Announced
Alex Aravanis.
Sapphire Energy, San Diego, CA, USA.
No Abstract

208-Wkshp
Aqueous Processing of Cellulosic Biomass for Biological Production of Sustainable Transportation Fuels
Charles Wyman.
Bourns College of Engineering, University of California, Riverside, Riverside, CA, USA.
Cellulosic biomass including agricultural and forestry residues, substantial fractions of municipal solid waste, and grasses and woody plants grown for energy provide the only low cost and abundant resource that could be used for sustainable production of liquid transportation fuels on a large scale. The challenge is to develop low cost processing technologies to convert this inexpensive resource into viable transportation fuels. Biological conversion systems can apply the power of modern biotechnology to dramatically reduce the costs of converting cellulosic biomass to fuels, and ethanol represents a particularly desirable product in the near term because of its excellent fuel properties, widespread use, and compatibility with biological conversion systems. Ethanol use can also build from commercial experience gained with ethanol made from starch crops in the United States and cane sugar in Brazil. The most expensive steps in biological processing are associated with breaking down the cellulose and hemicellulose fractions of biomass to release sugars, with pretreatment particularly critical to achieving low costs and high yields Our research focuses on improving the understanding of aqueous pretreatment and its interactions with enzymatic hydrolysis with the goal of reducing costs. This presentation will summarize comparative data on pretreatment technologies, developing and applying a high throughput miniaturized approach to pretreatment and hydrolysis, and continuous fermentations to reduce enzyme use. Once successfully introduced into the marketplace, fuels from cellulosic biomass can have powerful and unique strategic, environmental, and economic benefits.

209-Wkshp
Replacing Fossil Oil with Plant Oils - for What?
Sten Stymne.
Dept. of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Alnarp, Sweden.
The value of the products and chemicals made from the 10% of fossil oil that is used as feed stock in the chemical industry is the same as the value of all fuel made from the remaining 90%. Using plant oil instead of fossil oil as feed stock for the materials and chemicals could capture much of this added value, if the plant molecules are optimized *in-planta* for the end use and thereby minimizing the downstream processing costs. I have a vision that within 20 years time replace 40% of the fossil oil used in the chemical industry with renewable plant oils, whilst ensuring that growing demand for food oils is also met. This will require a trebling of global plant oil production from current levels of 135 MT to about 400 MT annually. Realisation of this potential will rely on application of plant biotechnology to (a) tailor plant oils to have high purity (preferably > 90%) of single desirable fatty acids, (b) introduce unusual fatty acids that have speciality end-use functionalities, (c) increase plant oil production capacity by increased oil content in current oil crops, and conversion of other high biomass crops into oil accumulating crops. Using plant oils in the chemical industry will not only enable replacement of fossil oil but will, at least equally importantly, enable substantial overall energy savings and generate added-value for agricultural products that cannot be captured by using them